<Preliminary>Pre-treatment of Oil Palm Empty Fruit Bunch by White-rot Fungi for Enzymatic Saccharification

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Preliminary

Pre-treatment of Oil Palm Empty Fruit Bunch by White-rot Fungi for Enzymatic Saccharification

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Introduction

Agricultural and forestry wastes are raw materials that can be utilized for cellulose production, papermaking, and transformation into value-added products. For enzymatic conversion of lignocellulosic materials to fermentable sugars, network of lignin must be decomposed to enhance their susceptibility to enzymatic hydrolysis[1]. There are a number of pre-treatments for this purpose. Biological treatments using white-rot fungi are one alternative to chemical degradation of lignin. Empty fruit bunch (EFB) of oil palm *(Elaeis guineensis)* is a residue of palm oil production in Indonesia, Malaysia, and the rest of Southeast Asian countries[2]. EFB is a potential resource that can be utilized for the production of useful materials, but its industrial applications are still limited. In this research, the authors aimed at production of fermentable sugars from EFB by using pre-treatments with white-rot fungi. Effects of the fungal treatments on saccharification of EFB were evaluated, together with those of beech wood.

Materials and Methods

Three white-rot fungi, *Dichomitus squalens* (CBS 432.34), *Ceriporiopsis subvermispora* (FP 90031) and *Pleurotus ostreatus* (O-48) were cultivated at 28°C for 2–8 weeks in 200 ml Erlenmeyer flask containing 5.0 g air dried meal (30–60 mesh EFB or beech wood) and 15 ml a solution containing 0.5% glucose, 0.31% polypeptone, 0.01% yeast extract, 0.00241% $MnSO_4$, 1.0 ml Kirk's salt, and 0.5 ml sodium succinate buffer (20 mM, pH 4.5). Lignin and holocellulose content were analyzed by Klason and Wise methods, respectively. Monosaccharide content was analyzed as alditol acetates on a Shimadzu Gas Chromatograph GC-17A. Enzymatic hydrolysis of the decayed materials was carried out in a reaction mixture (5.0 ml) containing 0.25 g of the substrates, 11.2 FPU of cellulase (Meicellase from Meiji Seika), and 5.0 ml of sodium acetate buffer (20 mM, pH 4.5). Glucose was determined by GOD method.

Activities of lignin-degrading enzymes (manganese peroxidase, lignin peroxidase, and laccase) were assayed spectrophotometrically. Activity of lipoxygenase (LOX) was measured with an oxygen electrode using linoleic acid as a substrate. A JEOL-JSM-5310 Scanning Electron Microscope (SEM) was used for observation of cell wall structures after fungal decay.

Results and Discussion

D. squalens degraded lignin most rapidly among the three fungi. After 8 weeks, weight loss of the lignin and holocellulose in beech wood reached 75.9%, and 49.9%, respectively. The fungus also delignified EFB. After 8 weeks, weight loss of lignin and holocellulose in EFB was 25.7% and 22.8%, respectively.

When decayed EFB was hydrolyzed with cellulase, saccharification yields of the EFB increased with increasing incubation time. Saccharification of EFB decayed by *D. squalens* was much higher than the other two fungi (Fig. 1).

Activities of MnP and laccase were found in the decayed EFB. However, activity of LiP was not detected. Laccase activity of *P. ostreatus* on EFB reached maximum, (44.7 U/flask) at day 8. *D. squalens* also produced 19.4 U/flask of laccase on EFB at day 12. *D. squalens* also produced 24.3 U/flask of MnP on EFB at day 16 (Fig. 2).

LOX activity was found in all of the decayed EFB. The highest activity was found in EFB decayed by *P. ostreatus* at day 16 (52 mU/flask).

In general, vascular bundles consist of xylem, phloem, axial parenchyma and fibers. Parenchyma is the main representative of the ground tissue system[3]. The existence of ground parenchyma tissue is embedded around the vascular bundles. SEM demonstrated that *P. ostreatus* degraded cell walls and middle lamelae of beech during 8 weeks. *D. squalens* and *C. subvermispora* preferentially attacked middle lamelae without intensive

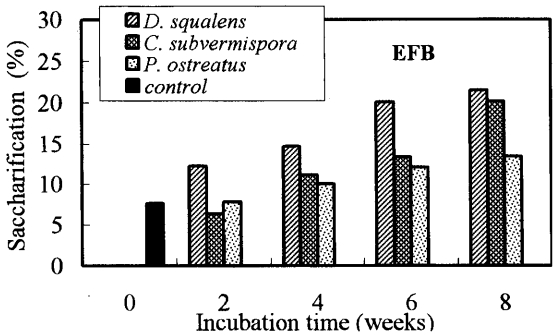

Fig. 1. Saccharification of EFB after fungal treatment (reaction time: 48 h; based on original EFB).

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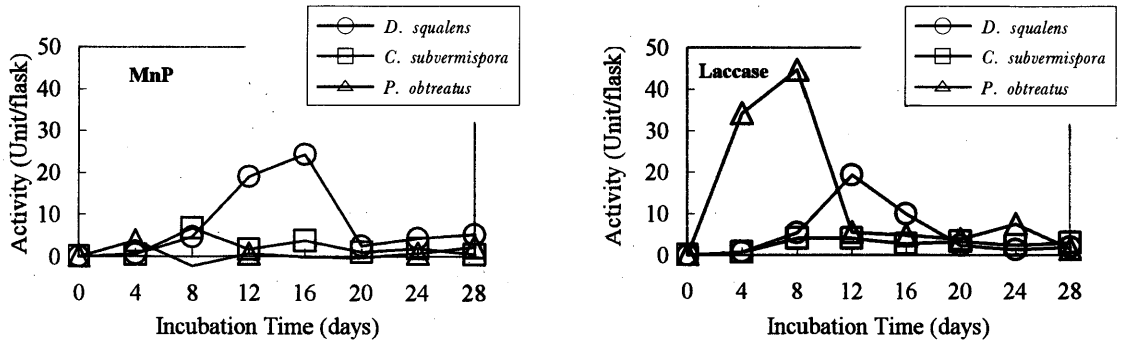

Fig. 2. Production of MnP and laccase by white-rot fungi on cultivation with EFB.

morphological damage of the cell walls. In EFB decay by three white-rot fungi, there was no severe damage of the cell walls of phloem and fibers. Only a few hyphae grew inside of the vascular bundles. However, all three white-rot fungi intensively attacked outside of the vascular bundles, parenchymatous ground tissues.

In summary, pre-treatments by white-rot fungi are effective for saccharification of EFB. EFB was also found to be a useful cultivation medium for the production of laccase and MnP. Screening of useful fungal strains and survey of optimum culture conditions will lead to practical applications of the biological treatments to produce various fermentation products from the residual lignocellulosics.

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